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Begin the article with a short summary.

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Treatment of Acute Leukemia With 5-Azacytidine (NSC-102816)^{1,2,3}

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SUMMARY

5-Azacytidine was administered to 28 patients with acute leukemia and four patients with chronic myelogenous leukemia in blast cell crisis, all but one of whom had received extensive prior therapy. Four complete and four partial remissions were achieved in 29 patients evaluable for response. The median duration of complete remission was 19 weeks. Nausea, vomiting, and diarrhea were common. Hypotension following administration of the drug occurred in four patients, and in one case this was fatal.

[Cancer Chemother Rep 57:319-323, 1973]

5-Azacytidine is a pyrimidine nucleotide analog produced microbiologically by a species of *Streptoverticillium* which was initially isolated in Czechoslovakia (1). It closely resembles cytidine in chemical structure and is incorporated into ribonucleic acids, affecting both DNA and RNA synthesis (2). In vitro, it inhibits RNA synthesis by the blocking of orotidylic acid decarboxylase (3). The drug is converted to a nucleotide by uridine kinase and is subsequently phosphorylated and incorporated into RNA; this modified structure inhibits protein synthe-

sis (4). The diphosphate is a substrate of the nucleotide reductase and, subsequently, DNA pronase; thus, the drug is also incorporated into DNA. In cell culture systems, there is evidence suggesting that the drug has some cell-cycle-nonspecific activity as well as cell-cycle-specific activity (5). In animal systems, the mechanism of resistance is a reduction in the activity of uridine kinase and a subsequent reduction in the conversion of 5-azacytidine to 5-azacytidine-5'-phosphate.

5-Azacytidine has been reported to be active in tumors resistant to cytosine arabinoside, vincristine, 6-mercaptopurine, 5-hydroxypicolinaldehyde thiosemicarbazone, and BCNU. In experimental animals, it is synergistic with both L-asparaginase and 6-thioguanine (5).

Previous studies have shown that this drug is active in a number of solid tumors and in acute leukemia. The original reports from Czechoslovakia showed encouraging results in the treatment of acute leukemia; however, these patients received other drugs known to be active against leukemia, and assessment of the activity of 5-azacytidine alone is difficult (6). Activity against solid tumors has been shown in phase I and II studies with objective responses

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³ 5-Azacytidine was supplied by Cancer Therapy Evaluation, Division of Cancer Treatment, NCI. Chemical nomenclature, NSC numbers, and CAS registry numbers for all compounds mentioned in this paper are listed in the List of Compounds at the end of the text.

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occurring in patients with cancer of the breast, in patients with melanoma, and in patients with cancer of the colon (7).

This study was designed to test the efficacy of 5-azacytidine when used alone in the treatment of patients with acute leukemia (regardless of age or cell morphology) who had active disease following chemotherapy with other anti-leukemic agents.

MATERIALS AND METHODS

A total of 32 patients were entered into the study; 31 of these patients had had extensive prior therapy, given either in combination or sequentially. One patient with chronic myelogenous leukemia in blast crisis had had no previous chemotherapy. The patients ranged in age from 1 to 72 years (median age, 27 years), and 20 of the 32 patients were male. Eighteen of the patients had acute myeloblastic leukemia, four had chronic myelogenous leukemia in blast crisis, and ten had acute lymphoblastic or undifferentiated leukemia.

The initial starting dose of 5-azacytidine was $400 \text{ mg/m}^2/\text{day} \times 5$; it was selected after a phase I study in patients with solid tumors had shown this dose to be well tolerated.⁵ The drug was supplied as a white powder in 50-mg vials and was reconstituted in water and mixed with 50–200 ml of 5% dextrose in water immediately prior to use. It was administered intravenously (iv) for 15–30 minutes daily for 5 days. Twenty-seven of the 32 patients received an initial starting dose of 400 mg/m^2 , three received a starting dose of 300 mg/m^2 , and two received a starting dose of 150 mg/m^2 . The second course was administered on Day 15, and doses were modified as required. The dose was escalated in the second course for five patients and reduced for four patients. One patient received 400 mg/m^2 for the first course, 500 mg for the second course, and 600 mg for the third course prior to achieving complete remission. For nine patients, the initiation of the third course was delayed for 3 weeks because of bone marrow hypocellularity (less than 5%). Two patients required the reinstitution of 5-azacytidine therapy earlier (one on Day

11 and one on Day 14) because of increasing disease in the bone marrow and peripheral blood.

Complete remission is defined as less than 10% blasts and promyelocytes in the bone marrow and less than 5% blasts, a hemoglobin value greater than 11 g/100 ml , a granulocyte count greater than 1000 cells/mm^3 , and a platelet count greater than $100,000 \text{ platelets/mm}^3$.

RESULTS

Of the 32 patients who were entered into this study, 29 were evaluable. Three patients were not considered to be evaluable for response because they were withdrawn from the study immediately after the first course of chemotherapy (at the parents' request). Eight patients had a hematologic response, four achieving complete remission. Seven patients died within the first 3 weeks after the start of chemotherapy. Eight patients had less than three courses of chemotherapy and were considered to have had inadequate trials. Six patients failed to respond.

The response rate in patients who were evaluable for response was 28% (eight of 29 patients), with complete remission being achieved in 14% (four of 29 patients) (table 1). The response rate for those patients receiving adequate trials of chemotherapy was 57%, and the complete remission rate for those receiving adequate trials was 28%.

When the response is broken down by diagnosis (table 2), it can be seen that most of the responses occurred in patients with acute myeloblastic leukemia. Only one patient with acute lymphoblastic or acute undifferentiated leukemia achieved a complete remission. None of the patients with acute lymphoblastic leu-

TABLE 1.—Response to treatment with 5-azacytidine

No. of responses/No. of evaluable patients	8/29 (29%)
No. of complete responses/No. of evaluable patients	4/29 (14%)
No. of responses/No. of adequate trials	8/14 (57%)
No. of complete responses/No. of adequate trials	4/14 (28%)

⁵ McCredie KB, and Gottlieb JA. Unpublished observation.

TABLE 2.—Diagnosis and response to treatment with 5-azacytidine

Diagnosis	Complete response	Partial response	Total No. of responses/ No. of patients with diagnosis (%)
Acute myeloblastic leukemia	3	4	7/18 (39)
Acute lymphoblastic leukemia	1	0	1/10 (10)
Chronic myelogenous leukemia-blast crisis	0	0	0/4 (0)

kemia had a partial response, and none of the four patients with chronic myelogenous leukemia in blast crisis responded.

The median duration of therapy for the evaluable patients was 42 days, with a range of 10–294 days. The median duration of therapy for those patients who failed to respond to chemotherapy was 30 days, with a range of 10–84 days. The median duration of response for those patients who responded to chemotherapy was 173 days, with a range of 59–294 days. In the four patients who achieved complete remission, the durations of chemotherapy were 166, 181, 247, and 294 days. The median duration of complete remission was 136 days (range, 73–200 days).

Maintenance therapy with intermittent doses of 5-azacytidine was difficult because of profound and often prolonged myelosuppression. Three of the four patients who achieved complete remission had delays of 3 months or more in the introduction of maintenance therapy because of severe granulocytopenia and thrombocytopenia. The myelosuppression in these patients was complicated by infection which required hospitalization and intensive therapy with antibiotics and leukocyte transfusions. The delays in the start of remission maintenance therapy were 90, 102, and 114 days. Maintenance therapy was started after recovery from this myelosuppression at the time or shortly after complete remission was observed.

In two patients, platelet transfusions were required for 25 of the 102 days and 32 of the 114 days. Both of these patients had granulocytopenia with total peripheral granulocyte counts of less than 1000/mm³ for 40 and 42 days, re-

spectively. Both patients experienced episodes of septicemia, one with *Klebsiella* sp. and *Candida albicans*, and the other with *Aeromonas* sp. These conditions were eventually controlled with appropriate antibiotic therapy and supportive care with granulocytes prior to the recovery of normal peripheral granulocyte counts.

The third patient had a delay of 3 months before the next course of chemotherapy, and required hospitalization because she had a fever of unknown origin. Positive blood cultures were not obtained; however, she had infected thrombosed hemorrhoids, and a pure growth of *Proteus mirabilis* was isolated. She responded satisfactorily to appropriate antibiotic therapy and white blood cell transfusions.

In the fourth patient, there was a 2-month delay between the second and third courses of chemotherapy, and hospitalization was required for 3 weeks because of thrombocytopenia and granulocytopenia. Platelet transfusions were started on Day 12, and prophylactic antibiotic therapy was started; no positive cultures were obtained despite persistent fever. After the patient recovered, the fourth course of chemotherapy was started in 45 days without marked myelosuppression, and the fifth course was started 30 days later.

All patients treated with 5-azacytidine experienced nausea, and most patients experienced vomiting and diarrhea (table 3). These symptoms were severe enough to cause marked anorexia in the majority of patients, and a few patients required iv therapy to prevent dehydration. Sixty percent of the patients receiving a rapid iv infusion of 5-azacytidine experienced fever with elevations in body tem-

TABLE 3.—Toxic effects of 5-azacytidine

Toxic effect	Rate of occurrence (%)
Nausea	100
Vomiting	90
Diarrhea	65
Fever	60
Bone marrow hypoplasia	25
Hypotension	12

perature to 104 F. The fever usually occurred 1–4 hours after the administration of the drug and continued each day for the duration of the course. However, body temperatures fell to normal levels within 24 hours of the completion of the course. On the whole, the drug was better tolerated with the second course of chemotherapy; however, nausea, vomiting, diarrhea, and fever were still major side effects. Severe bone marrow hypoplasia resulting in profound peripheral pancytopenia and the resultant complications of infection and hemorrhage occurred in one quarter of the patients and prevented the administration of the second course in most patients. Hypotension associated with the administration of the drug was seen in four patients; in one patient, it was associated with oliguric renal failure and death.

In the patient that died there was a direct relationship between the infusion of 5-azacytidine and the development of hypotension, tachypnea, and cyanosis. Although treatment was discontinued, the blood urea nitrogen continued to increase, and severe diarrhea associated with the administration of 5-azacytidine caused considerable electrolyte imbalance. Despite vigorous supportive care, this patient died 5 days later. Autopsy showed evidence of disseminated *Candida tropicalis* infection in the kidneys, adrenal glands, gastrointestinal tract, and lungs; this infection was unsuspected prior to the patient's death.

DISCUSSION

In this study, 5-azacytidine was used to treat 32 patients with acute leukemia. The patients who were selected for this study were in all

age groups and, with the exception of one patient with chronic myelogenous leukemia in blast crisis, had advanced disease which either had failed to respond or had relapsed with conventional forms of combination chemotherapy. The drug has been shown to be considerably active in patients with acute myeloblastic leukemia, as well as in patients with acute lymphoblastic disease. Although activity was not seen in patients with chronic myelogenous leukemia in blast crisis, all but one of these patients had had extensive prior therapy, and the course in all cases was complicated by marked myelosuppression and death.

The toxic effects experienced by this group of patients were marked (particularly the myelosuppression), and maintenance of remission with this drug alone is complicated. The toxic effects have been lessened by a reduction in starting dose from 400 to 300 mg/m²/day × 5, each daily dose being divided into three daily infusions given over 30 minutes. When given by this regimen, the drug appears to be better tolerated, and the hypotension, nausea, vomiting, and diarrhea appear to be lessened.

With the synergism shown in laboratory animals, 5-azacytidine may well have a role in future combination chemotherapeutic regimens for the treatment of acute leukemia of both adults and children. These combinations may be better tolerated for remission induction and may also allow for more effective remission maintenance therapy.

LIST OF COMPOUNDS

- L-Asparaginase: NSC-109229; CAS reg. No. 9015-68-3
- 5-Azacytidine: NSC-102816; CAS reg. No. 320-67-2; 5-triazin-2(1H)-one, 4-amino-1-β-D-ribofuranosyl-
- BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-
- Cytosine arabinoside: NSC-63878; CAS reg. No. 69-74-9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride
- 5-Hydroxypicolinaldehyde thiosemicarbazone: NSC-107392; CAS reg. No. 19494-89-4
- 6-Mercaptopurine: NSC-755; CAS reg. No. 6112-76-1; purine-6-thiol, monohydrate
- 6-Thioguanine: NSC-752; CAS reg. No. 1832-72-0; purine-6-thiol, 2-amino-
- Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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